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Direct Introduction of Milk Sample in FIA System for Phosphorus Determination

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Abstract

This work regards the determination of phosphorus in milk using a flow injection analysis (FIA)system. The determination is based on well known reaction between phosphate ions and an ammonium molybdate reagent, originating the ammonium phosphomolybdate complex, which is then reduced to form a blue compound of molybdenum that can be spectrophotometrically determined at 700 nm. Innovation of system is the on-line sample treatment, using nitric acid 5% (v/v) in flow causing the precipitation of proteins which were separated in a filter coupled to FIA. The method was compared with the well-established mineralization procedure, employing microwave assisted digestion procedure and the results were in agreement (paired t-test, p=0.05). The precision expressed as coefficient of variation for triplicate measurements was always lower than 10%. The limit of quantification was 0.572 mg L^{-1}.

Keywords: FIA, phosphorus, milk, sample preparation.

1. Introduction

Milk is very important food as source of proteins and minerals which are essential to the human organism. To children, young adults and also senior adults, milk contributes to bone structure, as well as endocrine function and muscular formation [1].

Phosphorus and calcium are among the main minerals present in milk. These are both related to bone and tissue development. Particularly, phosphorus participates in almost all metabolic processes of the human organism. One of the main functions is to help in the pH control, and energy storage in the formation of ATP [2].

Phosphorus is present both in the inorganic and organic forms as casein and casein phosphopeptide [3] in the milk. The concentration of phosphorus in skim milk is *ca.* 932 mg L^{-1} and in whole milk is *ca.* 908 mg L^{-1} [4]. It is possible because when sampling the whole milk fraction of sampled volume if fat, which has less phosphorus than an equivalent volume of skim milk.

The routine determination of ion concentrations, such as Ca^{2+}, PO_4^{3-} and Cl^-, in milk is very important in the industry. The anions Cl^- and PO_4^{3-}, for example, affect the quality of the fermentation and coagulation, and influence on the flavor and texture of the final product [3].

The determination of phosphorus is usually done by classical analytical methods, gravimetric or volumetric. However, before the determination, sample preparation is usually necessary. An ideal procedure for sample preparation should be simple, to use small volumes of reagents, fast and, of course, to solubilize all the sample constituents' [5]. The conventional procedures for preparation of milk samples involve the conversion of phosphorus-containing compounds to phosphate using digestion in acidic aqueous solutions [6] with conventional heating or microwave assisted heating can be considered adequate to different kinds of matrix.

Among the instrumental techniques for the determination of phosphate, may be mentioned the spectrophotometry based on the reaction of phosphate with ammonium molybdate in acidic medium [7]. This reaction has been used to determine phosphorus in different matrices, being fundamental a prior efficient sample preparation originating phosphate, to enable the chromogenic reaction with subsequent readings of absorbance. In this case, is assumed that total phosphorus was determined once that all phosphorus present in the sample was converted to phosphate due to the acid digestion.

The Flow Injection Analysis (FIA) systems have been widely used to improve the analytical performance of the determinations, mainly spectrophotometric. These systems are attractive alternatives because their versatility and possibility of coupling to various detection techniques, such as atomic absorption spectrometry, atomic emission spectrometry, chromatography, molecular absorption spectrometry. [8 -10]. In FIA, samples and reagents are inserted into a carrier stream, driven by a peristaltic pump at a flow rate previously studied. Therefore, a concentration gradient is generated in the system as a result of a dispersion process. The main advantages are: low consumption of reagents and sample, and high analytical throughput [3].

The present work describes a FIA system to determine phosphorus in milk samples which were directly introduced in the system, with an on-line sample preparation. A filtration system was accomplished to FIA for separation of milk proteins after its acid hydrolysis. The well know reagents: ammonium molybdade and ascorbic acid to form the molybdenum blue were employed. The study also shows a comparison between the proposed system and a well established sample preparation technique, using microwave assisted digestion for milk samples.

2. Experimental

2.1. Apparatus

The FIA system consisted a peristaltic pump (Ismatec IPC), to propel the fluids through polyethylene tubes (i.d. 0.8 mm); an injector-commutator and an autosampler built in our laboratory; a Spectrophotometer (Biospectro, SP-220), 1 cm optical path flow cell, to detection; a PCL-711(Advantech) interface and software in Visual Basic language for control and acquisitions of data [11]. The filtration system, described elsewhere [12] was accomplished to FIA system for on-line sample preparation.

As comparative method an acid digestion using a microwave oven (Millestone, Etho plus) with closed vessels in which, was used.

2.2. Sample reagents and solutions

All reagents and solutions were prepared with purified water in Milli-Q (Millipore) system (18 MΩ cm).

The skim milk and whole milk samples were obtained in local market. Before their introduction in the FIA system, the samples were only diluted (1:100) with deionized water.

In the FIA system, ascorbic acid (Isofar), 1% (w/v), nitric acid (Merck), 5% (w/v), ammonium molybdate (Vetec), 1% (w/v), and nitric acid 0.5 mol L^{-1} were used. The phosphate stock solution (1.000 mg L^{-1}) was prepared with KH_2PO_4 (Proquimios).

In order to verify the sample preparation efficiency, the same samples were digested into microwave oven vessels in which was added 0.5 mL of sample, 4.0 mL of HNO_3 and 1.0 mL deionized H_2O_2.

The heating program in microwave oven was on two steps: first was done the ramp heating up to 200 ^oC with 1000 W of power during 10 min, and after was applied the same power to hold 200 ^oC for more 20 min.

2.3. FIA Manifold

The FIA manifold is shown in Figure 1. In the position A the sample loop (SL) is filled and, simultaneously, a clean of filtration system (F) was done. In the position B, the sample is transported until confluence X_1, in which the HNO_3 for protein precipitation and phosphorus hydrolysis is added (R_1). These precipitates were separated in the filter system (F) (which contained glass fiber of 0.45 mm membrane (Milipore) sliced with 1 cm of diameter) , and phosphorus hydrolyzed was transported in solubilized fraction. Following, the reactions with molybdate (R_2) and ascorbic acid (R_3) occur in X_2 and X_3.confluences, respectively. Finally, the measurements in spectrophometer (700 nm) were done and the sample was discarded to waste (W).

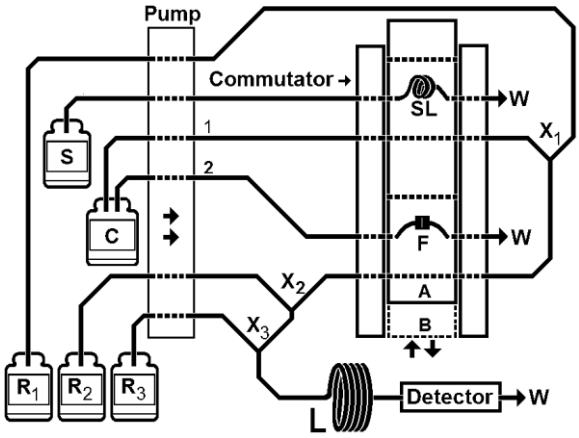

Fig. 1: FIA manifold: S – sample, (6 mL min-1); C1 – carrier stream of water (3.5 mL min-1); C2 – clean stream of water (5 mL min-1); R1 – nitric acid 5% w/v (3.5 mL min-1); R2 – ammonium molybdate (2.1 mL min-1); R3 – ascorbic acid (2.1 mL min-1); SL – sample loop (5 cm); F – filter; L – reactor loop (230 cm); W – waste and X1, X2 and X3 – confluences.

3. Results and discussion

Milk is a very complex matrix which contents several proteins and others organic compounds. Therefore, for an adequate accuracy the standard addition calibration was used, and phosphorus in the concentrations of 10.0, 20.0 and 30.0 mg L^{-1} were added to diluted sample (1:100).

The good linearity of analytical curves can be confirmed through the linear correlation coefficient (r^2 were always higher than 0.991).

Figure 2 shows one example of an absorbance signal for a milk sample, where it can be observed the good precision of measurements with coefficient of variation always lower than 10%. All the measurements (about 150), in this work, were done with the same filter, thus can be estimated about 300 measurements with no change of the filter.

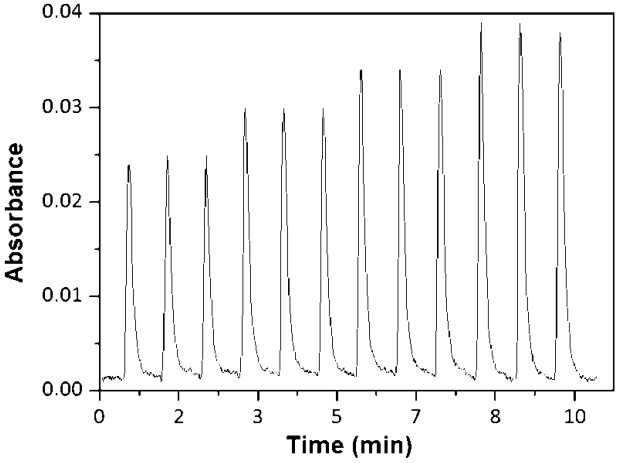

Fig.2: Signal recording of a sample and an analytical curve.

The method presented a high analytical frequency (about 70 readings per hour). Thus, the proposed method can be considered fast (sample preparation and determination are executed in only one step, with duration of less than one minute) when compared to the conventional method, in which only for the sample preparation step, using microwave assisted digestion, it is necessary about twenty minutes.

Analytical parameters, Limits of Detection and Quantification (LOD and LOQ, respectively) were determined and are presented in Table 1. Considering the low LOD obtained, it can be affirmed that the method is useful for phosphorus determination in milk samples, once that the usual concentration found in this type of sample is around of 1,000 mg L^{-1}.

Table 1. Phosphorus concentration.

Sample	Standard Method (mg L^{-1})	Proposed Method (mg L^{-1})
1*	1315 ± 2.7%	1239± 0.7%
2*	1064 ± 3.4%	1151± 6.6%
3*	981 ± 0.0%	952± 9.9%
4*	1189± 3.0%	1206± 0.9%
5**	1065± 3.4%	1087± 6.5%
6**	1028± 3.2%	973± 3.5%

* whole milk ** skim milk

To confirm sample preparation feasibility, a comparison with well established microwave oven assisted digestion was done. Table 2 summarizes the results obtained for the samples analyzed using the proposed method as well as a standard procedure. The results were in agreement at a confidence level of 95% (paired *t*-test). The accuracy was checked with addition/recovery tests, and the recovery values were around 98%.

When the phosphorus is in the complex form and has strong bonds with organic compounds, a digestion procedure as microwave –assisted wet acid digestion is necessary to form phosphate in solution. On the other hand, when the phosphorus is weakly bounded, only gentle procedure as hydrolysis at room temperature can be adequate. The similar values obtained, in this work, with microwave-assisted acid digestion and FIA system, indicate that the phosphorus presents in milk is weakly bounded, or no bonded (inorganic form).

Table 2. Analytical Parameters.

Parameters	Values
Limit of detection (mg L^{-1})	0.172
Limit of quantification (mg L^{-1})	0.572
Correlation coefficient (r^2)	0.991
Linear response range (mg L^{-1})	0.572– 30.0

4. Conclusions

The proposed method presented satisfactory results for phosphorus determination in milk samples, and can be used in routine analysis because it is simple and easy for implementation.

The main advantages of the proposed method are: the high analytical frequency with about 70 readings per hour and the low sample and reagents consumption. It can be mentioned that the presented method is in agreement with green chemistry, once it generates low waste volumes.

Also it can be supposed that phosphorus presents in milk is weakly bounded to proteins because only a mild hydrolysis is enough for releases this species.

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